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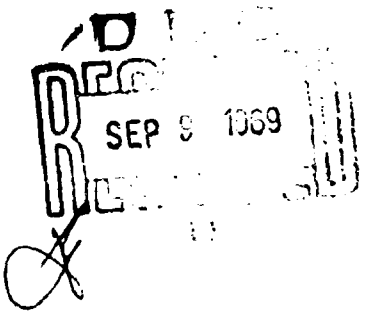

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CLINICAL-MORPHOLOGICAL AND IMMUNOLOGICAL PARALLELS IN GUINEA PIGS INFECTED WITH VACCINE AND VIRULENT STRAINS OF R. BURNETTI

[Following is the translation of an article by O. S. Gudima and A. M. Igonin (Moscow), published in the Russian-language periodical Arkhiv Patologii (Archives of Pathology) 24(8): 50-55, 1962. It was submitted on 20 May 1961.]

Following a single administration to animals, live vaccines cause a prolonged and intensive immunity.

In the opinion of some investigators [18, 19, 23] the interaction of the macroorganism with a vaccine strain apparently represents a principally new process which has no analogies in the science of infectious diseases. In the opinion of others, following the immunization of animals with attenuated vaccine strains of microbes an infectious diseases with an unusual course develops. The entire process, both local and general, bears a specific weakly expressed benign nature with an outcome of complete clinical and anatomical recovery. This position has been confirmed relative to tuberculosis vaccine [15, 16], antiplague, brucellosis [4, 13], tularemia [23, 24], typhus [14], and anthrax [25] vaccines. This same opinion is maintained by I. V. Davydovskiy [6].

The purpose of the present investigation was a parallel clinical-morphological and immunological study of changes in the organism of guinea pigs which had been infected with vaccine and virulent strains of R. burnetti.

Material and Method of Investigation

The investigation was conducted on guinea pigs weighing 200-300 g. Two groups of animals (40 pigs in each) were infected subcutaneously in the left inguinal area. Guinea pigs of the first group received 10,000 cu * each of causative agent of a vaccine strain (strain BD-1 [19]), and animals of the second group - 10,000 cu each of virulent causative agent (original "Grit" strain).

* cu - conditional unit, i.e., the least amount of causative agent causing a positive serological reaction in 50% of infected guinea pigs.

After infection the animals were placed in pairs in glass jars.

Temperature was checked daily on 10 guinea pigs in each group.

For obtaining morphological data animals were sacrificed (two guinea pigs each) on the 2nd, 4th, 5th, 8th, 10th, 12th, 14th, 16th, 20th, 25th, 30th, 40th, 60th, and 90th days after infection. Fragments of internal organs - brain, heart, lungs, liver, kidneys, spleen, testes, regional and general mesenteric lymph nodes, and subcutaneous connective tissue in the area of infection - were fixed in tsenkerformol /*/, absolute ethyl alcohol, and enclosed in paraffin. Sections were stained with azure II-eosine and methyl green - pyronine.

/*/ Trans. Note: This word has not been identified in available sources.

In these same periods blood was taken from the animals for serological investigation in the complement fixation reaction, which was set up by the method described by P. F. Zdrodovskiy and Ye. M. Golinevich /10/.

Data from Clinical and Serological Investigations

In guinea pigs which were infected with the vaccine strain no noticeable increase in temperature was observed. In animals infected with the virulent strain of rickettsia, starting with the 10th day a febrile reaction is noted which achieves a maximum by the 10--12th day and lasts up to the 16th day (Fig. 1).

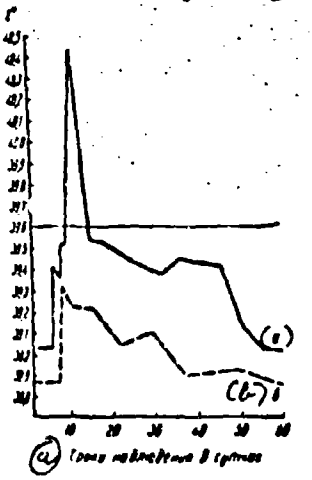

Fig. 1. Febrile reaction in guinea pigs, infected with virulent (a) and vaccine (b) strains of R. burnetti.
Key: (a) Period of observations in days.

At the site of infection in animals of both groups infiltrates appear on the 6th day. By the 11th day they reach a diameter of 1 cm and become thick. With the 16th day they are abated and disappear on the 20--25th day after infection.

In animals which were infected with the causative agent of a virulent strain complement-fixing antibodies are revealed in the blood serum on the 14th day after infection. Titers of antibodies continue to increase, reaching a maximum on the 20th day (1:2560). They remain on this level up to 40 days. After this period a slow lowering is observed in the titers of antibodies. On the 60th and 90th days their level is reduced to 1:320 (Fig. 2).

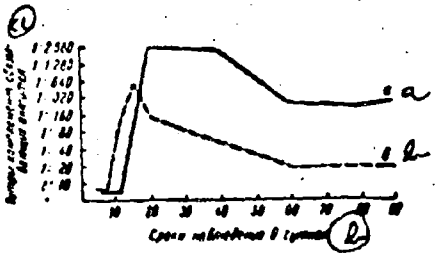

Fig. 2. Titers of complement-fixing antibodies in guinea pigs infected with virulent (a) and vaccine (b) strains of R. burnetti. Key: Titers of complement-fixing antibodies; (b) Periods of observation in days.

In guinea pigs which were infected with the vaccine strain of rickettsia an increase of titers of complement-fixing antibodies is noted starting with the 11th day, with a maximum on the 20th day (1:2560). After this period titers are reduced and by the 60th day reach levels of 1:10. They do not drop lower than this right up to the 90th day.

Results of Morphological Investigation

Macroscopically on the guinea pigs of both groups edema of the subcutaneous cellular tissue is revealed at the site of injection on the 1st day after infection. On the 6th day extensive hemorrhages are noted in the edematous cellular tissue, mainly around the infiltrate noted. The size and thickness of the infiltrates on the animals increase in the later periods. Muscles of the abdominal wall are involved in the inflammatory process, and in individual cases the peritoneum. Expressed adenitis of the regional lymph nodes and enlargement of the spleen are observed. In the lungs starting with the 4th day minute pneumonic foci appear and they increase rapidly. On the 6th day and later focal specific pneumonia is developed in all the animals. The heart and brain remain without apparent changes.

During microscopic investigation of infiltrates rickettsia are detected in the cells of connective tissue and extracellularly up to 10 days after infection. This period is characterized by the

development of plasma cells and a macrophagal reaction with the formation of gigantic multinuclear cells, sometimes containing segmentonuclear leukocytes. Plasma cells begin to appear with the 4th day and their number continues to increase up to 16--20 days. On the 25th day the infiltrates consist mainly of sharply pyroninophilic plasma cells at various stages of maturity. The phenomena described are inherent to the same degree to animals of both groups.

In the brain changes are noted only in the choroid plexus, where weak infiltrates of lymphoid cells are apparent around the vessels.

In the cardiac muscle, mainly around the vessels, in the majority of animals beginning with the 6th-8th day after infection insignificant infiltrates of polyblasts, lymphocytes, and plasma cells are revealed.

In the lungs increasing pneumonic phenomena are noted from the 1st day. In the cells and extracellularly rickettsia are detected in large numbers. Among the proliferating cells there are many plasma cells, which are especially apparent starting with the 16th day after infection of the animals, i.e., in the period of morphological restoration of pulmonary tissue.

In the liver numerous perivascular infiltrates of basophilic cells and macrophages are observed, and in the kidneys of all the investigated animals - multiple foci of cellular proliferation both in the cortical and medullary layers. Extensive cellular infiltrates, mainly from plasma cells, are visible in tissues of the pelvis. In the area of the infiltrates and around them the cells of renal epithelium are partially subjected to dystrophy. The presence of a large number of rickettsia in the protoplasm of epithelial cells is characteristic.

In the regional lymph nodes noticeable morphological changes are noted already on the 2nd day. They lead to the rapid drawing out of a large number of small lymphocytes into the sinuses and further into the lymph paths. Simultaneously there are signs of swelling and hyperplasia of cells of intersinus reticular tissue. Subsequently the hyperplasia of reticular tissue is accompanied by intensification of differentiation of its cells into plasma cells. Differentiation is distinctly apparent already starting with the 4th day after infection of animals. Up to the 10th day the plasmocellular reaction and hyperplasia of cells of intersinus reticular tissue proceed with particularly vigorous tempos and reach almost a maximum degree of expressiveness. In the majority of animals by this period the soft cords of the lumph nodes are made up almost without exception of plasma cells of a various degree of maturity.

In the period from the 10th to the 16th days the increase of hyperplasia of reticular tissue and the plasmocellular reaction take place relatively more slowly than in the incubation period.

After 16-20 days morphological symptoms of restoration of normal structure of the lymph nodes are noted: zones of lymphoid cells appear around the follicles and sinus cells are restored. The structure of lymphoid follicles is normalized only by the 30th--40th day. The number of plasma cells continues to remain large. Some reticular cells remain swollen right up to 90 days.

Difference in morphological changes in the regional and other lymph nodes is noticeable only up to 4 days.

In the spleen distinct morphological changes are noted starting with the 4th day after infection. By this time accumulations of basophilic (pyroninophilic) cells are noted, mainly around the trabeculae, blood vessels, and under the capsule. Their number increases rapidly and many of them differentiate into plasma cells. They fill up a large portion of the red pulp already by the 6th-8th day. Maximum accumulation of them takes place by the 10th-12th day. Between the 12th and 30th days no noticeable increase is observed in the number of plasma cells. After the 30th day the qualitative composition of plasma cells changes (in animals infected with the vaccine strain this is observed starting with the 20th--25th day). Among them mature forms start to predominate and degenerating forms appear along with a relative decrease in the number of transitional cells and plasmoblasts. Distribution of plasma cells is nest-like, and not diffuse, as this was during the incubation period and the febrile period.

Changes are noticeable in the cells of reticular tissue already on the 2nd day. On the 4th--6th day they are clear and are characterized by swelling and intensive multiplication of the reticular cells of the intersinus reticular tissue. At this time large accumulations of coarse polygonal cells appear. By the 10th--12th day hyperplasia reaches a maximum. Over extensive sectors hyperplastic tissue displaces all other cells with the exception of plasma cells. Starting with the 20th day the cells as if unswell, i.e., their is a decrease in their volume, their protoplasm becomes more basophilic (pyroninophilic). Between the cells intercellular spaces appear which are filled mainly with lymphocytes. But these changes do not set in simultaneously in all sectors of the spleen. With the 25th day restoration of the normal morphological structure of reticular tissue is expressed still more distinctly. However, it does not return completely to its initial condition even by the 19th day.

With the onset of hyperplasia of reticular tissue the lymphoid cells are driven out into the sinuses. Differentiation and maturation of lymphoid cells in follicles is inhibited considerably during the entire period of acute hyperplasia of reticular tissue.

Discussion

It is evident from what has been set forth that the incubation period in experimental animals is accompanied by a building up of morphological changes. The last two days of it are characterized by expressed perivascular infiltrates and a sharply expressed hyperplasia of reticular tissue of the lymph nodes and spleen. Noticeable hyperplasia of reticular cells begins early - on the 2nd--4th day after administration of the causative agent - and is accompanied by the formation of plasma cells out of them [11]. Consequently, already in the incubation period a response protective reaction to the causative agent begins to form and it can be determined by morphological methods of investigation.

In these tests antibodies begin to be determined in the blood serum of animals only in the very end of the incubation period and even in the period of onset of fever (with the virulent strain of rickettsia). But such a late appearance of complement-fixing antibodies in the peripheral blood apparently does not speak for their late formation. According to S. F. Vakarina and S. I. Ginzburg-Kalinina [3], antibodies are determined in the organs which produce them in quite high titers (1:10--1:80) already in 6 hours after the subcutaneous administration of an antigen stimulus to rabbits. G. A. Gurvich and G. V. Shumakova [5, 26], during a determination of antibodies in extracts from the regional lymph nodes, detected complete conformity between the production of immune antibodies and the plasmocytic reaction. Such a nature of data were obtained by Ehrich and Harris [28, 29].

In our investigation no differences were revealed in the degree of expressiveness of morphological changes in the organs of animals which were infected with vaccine and virulent strains. The absence of this difference is evidently connected with the use of large doses of causative agent for infection of the animals.

A similar degree of expressiveness of morphological changes in the guinea pigs of both groups in one case (virulent strain) was accompanied by expressed fever, and in the other there was no fever. The absence of fever in animals infected with the vaccine strain can be explained to some degree by the loss of pyrogenic properties by the causative agent. According to Wagner and associates [31], and also Wendt and Kim [32], the febrile condition is connected with the formation, in tissues and especially in leukocytes, of a pyrogenic factor which appears as a result of the interaction of endotoxin with tissue fluids and blood.

In connection with this it is necessary to point out the very intensive development of rickettsia in the organs and tissues of infected animals during the incubation period. According to Bengtson [27], by the end of the incubation period the spleen of infected

guinea pigs can contain 10^9--10^{10} infectious doses of causative agent. Liberation of the organism from the majority of rickettsia takes place in 8--11 days after infection, i.e., still in the incubation period and in the period of onset of fever [10]. Here the endotoxin of rickettsia is probably the cause of the development of fever.

What is the significance of the morphological changes found? As can be seen from the literature, reticular tissue responds differentially and specifically to stimuli of various nature. Non-antigenic stimuli (India ink, oil) do not cause noticeable morphological and functional reorganization of reticular tissue. They only cause local reactions which are known as reactions to a foreign body. Differentiation of cambiogenetic elements in this case is directed to the maturing of polyblasts and macrophages [7, 30]. Acute loss of blood leads to differentiation of reticular cells and cambiogenetic elements of areolar tissue into hemocytoblasts and blood cells [1, 2]. Following antigen stimulation the reticular cells and cambiogenetic elements of areolar tissue differentiate into plasma cells [11]. An antigen stimulus is a specific stimulus of "protective devices" of an organism [20].

At present many investigators connect the formation of antibodies in infected animals with the development of a plasmocellular reaction, primarily in organs with reticular tissue [8, 9, 21]. It has to be assumed that the degree of functional reorganization of protective devices, in the first order of which stand organs with reticular tissue, depends on the intensity of the influence of this specific stimulus.

In the present investigation the morphological changes described in the organs of infected guinea pigs can, in our opinion, be appraised as changes which are connected with the formation of immunity to the particular causative agent. These changes are characterized primarily by functional reorganization of cells of reticular tissue. They are physiological, since they are not connected with a "breakdown" of structure and function of the organs and tissues. The idea of the separation of immunological processes from general pathological processes was expressed for the first time by Ya. L. Rapoport [21]. Our experimental findings apparently confirm the correctness of this idea.

Conclusions

1. In guinea pigs a vaccine strain of R. burnetti does not cause a clinically expressed illness, but leads to the formation of specific complement-fixing antibodies, the titers of which are considerably lower (1:640) than during infection with the same dose of a highly virulent strain (1:2560).

2. Under our test conditions no difference was revealed in the degree of expression of morphological changes in the organs of guinea

pigs which were infected with virulent and vaccine strains of R. burnetti.

3. The morphological changes described can be characterized as connected with the formation of immunity to the particular causative agent.

Literature

1. Abrikosov, A. I., Strukov, A. I., Pathological Anatomy, Moscow, 1953, part 1, p 227.
2. Al'pern, D. Ye., Pathological Physiology, Moscow, 1954, p 309.
3. Vakarina, S. F., Ginzburg-Kalinina, S. I., In the book: Bases of Immunity, Moscow, 1956, p 247.
4. Vershilova, P. A., Kokorin, I. N., In the book: Problems of Infectious Pathology and Immunology, Moscow, 1954, issue 2, p 244.
5. Gurvich, G. A., Shumakova, G. V., Byull. eksper. biol., 1957, No 10, p 35.
6. Davydovskiy, I. V., A Study of Infection, Moscow, 1956, p 62.
7. Zavarzin, A. A., Selected Transactions, Moscow, 1953, vol 2, p 9, 33.
8. Zdrodovskiy, P. F., Klin. med., 1959, vol 37, No 10, p 13.
9. Idem., Vestn. AMN SSSR, 1956, No 3, p 43.
10. Zdrodovskiy, P. F., Golinevich, Ye. M., A Study of Rickettsia and Rickettsioses, Moscow, 1956.
11. Igonin, A. M., Byull. eksper. biol., 1959, No 8, p 110.
12. Idem., Ibid., 1960, No 12, p 65.
13. Kokorin, I. N., Vestn. AMN SSSR, 1957, No 3, p 41.
14. Idem., Vopr. virusol., 1959, No 3, p 272.
15. Kozlov, Yu. A., Chalisov, I. A., Byull. In-ta Tuberkuleza AMN SSSR, 1948, No 4, p 7.
16. Korshun, S. V., Dvizhkov, P., Gorokhovnikova, A., et al., Moskovsk med. zh., 1926, No 11, p 16.
17. Kravchenko, A. T., Zh. mikrobiol., 1948, No 7, p 7.
18. Idem., Ibid., p 12.
19. Kravchenko, A. T., Gudima, O. S., Milyutin, V. N., Vopr. virusol., 1961, No 3, p 300.
20. Pavlov, I. P., Complete Collection of Works, Moscow-Leningrad, 1946, vol 2, p 350.
21. Rapoport, Ya. L., Arkh. pat., 1957, No 2, p 3.
22. Timakov, V. D., Zh. mikrobiol., 1954, No 10, p 3.
23. Chalisov, I. A., Spasskaya, M. G., Arkh. pat., 1946, No 5-6, p 99.
24. Idem., Ibid., 1948, No 1, p 34.
25. Chalisov, I. A., Tamarin, A. L., In the book: "STN" Anthrax Vaccine, Moscow, 1946, p 114.
26. Shumakova, G. V., Gurvich, G. A., Byull. eksper. biol., 1958, No 11, p 66.